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Larval density dependence in *Anopheles gambiae* s.s., the major African vector of malaria

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Summary

1. *Anopheles gambiae* sensu stricto is the most important vector of malaria in Africa although relatively little is known about the density-dependent processes determining its population size.
2. Mosquito larval density was manipulated under semi-natural conditions using artificial larval breeding sites placed in the field in coastal Kenya; two experiments were conducted: one manipulating the density of a single cohort of larvae across a range of densities and the other employing fewer densities but with the treatments crossed with four treatments manipulating predator access.
3. In the first experiment, larval survival, development rate and the size of the adult mosquito all decreased with larval density (controlling for block effects between 23% and 31% of the variance in the data could be explained by density).
4. In the second experiment, the effects of predator manipulation were not significant, but again we observed strong density dependence in larval survival (explaining 30% of the variance).
5. The results are compared with laboratory studies of *A. gambiae* larval competition and the few other studies conducted in the field, and the consequences for malaria control are discussed

Keywords

Anopheles gambiae; density dependence; field experiment; mosquito

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Introduction

Mosquitoes in the genus *Anopheles* are the sole vectors of the human malaria pathogen, *Plasmodium* (Gilles, Warrell & Bruce-Chwatt 1993). Malaria is globally one of the most significant infectious diseases and has been estimated currently to sicken over 200 million people annually and causes about three quarters of a million deaths (WHO 2010) although a very recent study (Murray *et al.* 2011), using new methodologies, has suggested that the number of deaths are considerably higher: 1·2 (95% uncertainty range, 0·9–1·7) million people in 2010. There has been intensive research on how the toll of malaria can be reduced or eliminated, efforts that have focussed on both targeting the *Plasmodium* in humans and interventions designed to interrupt transmission by the mosquito (Gilles, Warrell & Bruce-Chwatt 1993). Vector-control measures have included habitat modification to remove larval breeding sites (Ijumba, Mosha & Lindsay 2002; Yohannes *et al.* 2005), spraying relatively long-lasting insecticides on the walls of huts and houses to kill female mosquitoes where they rest after a blood meal (Pluess *et al.* 2010), and the use of bed nets, especially those impregnated with insecticide, to protect sleeping people (Lengeler 2004). Recently, there has been growing interest in the possibility of using genetic manipulation to interrupt transmission, either by introducing a construct that reduces mosquito fitness and hence population size or by knocking out a gene that codes for a product essential for malaria transmission (Burt 2003; Sinkins & Gould 2006; Chen *et al.* 2007). Effective implementation of any mosquito-control strategy requires a good understanding of the vector's ecology. This article describes a field study designed to help understand population regulation in the most important African vector of malaria.

Malaria burdens are highest in Africa (Snow *et al.* 2005; Murray *et al.* 2011) where most transmission is dominated by members of the *Anopheles gambiae* and *Anopheles funestus* complexes (Hay *et al.* 2010; Sinka *et al.* 2010). *A. gambiae* mosquitoes are very efficient vectors because of the strong preference of some members of the complex to bite humans. The complex consists of seven morphologically identical species with subtly different ecologies (Coluzzi *et al.* 1979). The two members responsible for most transmission are *A. arabiensis* and *A. gambiae* sensu stricto (henceforth, we use *A. gambiae* to refer to this form), which tend to be found in dryer and wetter areas, respectively. *A. gambiae* has a very complex population structure, especially in West Africa, which is still not fully resolved (Riehle *et al.* 2010).

It is clearly important to understand the population ecology of African *Anopheles* mosquitoes to design and optimise control measures. Two critical questions are: (i) What are the density-dependent processes that contribute to population regulation in these insects? and (ii) Where in the lifecycle do they occur? Answers to these questions can help determine the strength of interventions required to interrupt transmission and which life-history stage may be the most efficient to target. Most mosquito ecologists have assumed, implicitly or explicitly, that density dependence occurs largely in the larval stage through competition for food. For example, the Ross–McDonald model (Ross 1911; Macdonald 1957) that describes vector-transmitted diseases, and many models based on it, supposes mosquitoes recruit to the adult stage at a constant rate that is equivalent to assuming perfectly compensating pre-adult density dependence. There is limited experimental support for density-dependent larval

competition. In laboratory experiments, high larval densities of *A. gambiae* have been shown both to decrease (Lyimo, Takken & Koella 1992) and to extend (Schneider, Takken & McCall 2000) development times, while their effects on adult size are complex and influenced by temperature (Lyimo, Takken & Koella 1992; Schneider, Takken & McCall 2000). Ng'habi *et al.* (2005) found evidence that crowding affected male mating success independent of the amount of food provided per larva. Service (1973, 1977) studied natural parasitism and predation in paddy fields on larval *A. arabiensis* – a member of the *A. gambiae* complex. Density dependence in *A. gambiae* has also been inferred from the statistical analysis of time-series data (Russell *et al.* 2011).

The most important recent study to look at density dependence in African malaria vectors experimentally in the field was undertaken by Gimnig *et al.* (2002). In two experiments, they manipulated the density of *A. gambiae* larvae in artificial breeding sites placed in the field in Western Kenya. They found a strong effect of density on development time and adult size but no significant effect on survival. Njunwa (1993; see also White *et al.* 2011) in an unpublished study manipulated larval densities and found mortality increased and then plateaued as larval densities rose.

Despite the great significance of *A. gambiae* to human well-being, we believe these studies are the only field experiments to explore larval density dependence. Here, we report a series of experiments in which *A. gambiae* densities and cohort structure are manipulated in the field in coastal Kenya using experimental methodologies based on Gimnig *et al.* (2002). We detected strong density dependent in larval competition, although of a form different to the earlier study, and we explore the reasons for these differences and the implications for the control of mosquitoes.

Materials and methods

Study Site

The study was conducted in the village of Jaribuni (03°37·3S; 039° 44·6E) approximately 50 km west of Kilifi in Kilifi County on the Kenyan coast north of Mombasa. The study site is approximately 400 m above sea level and has two wet seasons annually, a long rainy period in April–June and short rains in October–December; mean annual precipitation is 750–1200 mm. Temperatures range from 22 to 30 °C, and the average relative humidity is ~70%. The area around Jaribuni consists of scattered villages and households with subsistence farming and small-scale rearing of cattle, goat and poultry, with some plantations of sisal, coconut and cashew nuts. The rural population mainly lives in stick and mud-walled houses with roofs thatched with coconut leaves. Malaria epidemiology has been studied in this area for many years by workers from the KEMRI-Wellcome Trust Programme. The most common vector is *A. gambiae* (though with *A. arabiensis* increasing in frequency) with *A. funestus* also significant. Our experiments had ethical approval from KEMRI (the Kenyan Medical Research Institute).

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Artificial Larval Habitats

Experiments were conducted in artificial larval habitats designed to mimic the natural breeding sites of *A. gambiae*. Clay pots 35 cm in diameter and 13 cm deep were placed in shallow depressions in the ground approximately 9 cm deep in experimental plots fenced to exclude animals. The plots were unshaded and had good drainage to avoid flooding after heavy rain. Small holes (5 mm diameter) were drilled beneath the rim of the pot and covered with fine gauze net to allow water drainage without loss of mosquito larvae. Locally collected, sterilised mud (1 kg) was smeared around the inner surface of each pot, and five litres of filtered (but not sterilised) water from the River Jaribuni (normally a small stream immediately adjacent to larval breeding sites) was added to prepare the larval habitats. The water depth at the start of the experiment was 10 cm, and further water was added each day to replace that lost to evaporation.

Experimental Mosquitoes

The mosquitoes used in the experiments were obtained from a laboratory colony originally initiated from mosquitoes collected at Kisumu in western Kenya. They were reared in an insectary at the KEMRI-Wellcome trust research laboratory in Kilifi. On the days the experiments were set up, mosquito eggs were allowed to hatch in plastic containers and the first-instar larvae counted into vials, which were transported to the field site.

Experiment 1

In this experiment, the densities of a single cohort of larval mosquitoes were manipulated, and the consequences for development time, survival and adult size assessed. The experiment consisted of seven temporally separated blocks set up between November 2009 and August 2010: three in the dry season and four in the wet season. In each block, larval breeding sites were assigned randomly to five density treatments: initial densities of 32, 64, 128, 256 or 512 first-instar larvae. The total number of larval breeding sites varied across blocks because of variation in the supply of mosquitoes available from the rearing facility and was 25 in two blocks, 20 in three blocks and 15 in two blocks. The pots were covered with fine netting sufficient to exclude predators and oviposition by wild mosquitoes. Each day, the breeding sites were monitored, and any pupae present were removed using a pipette and taken back to the laboratory to be reared. The resulting adults were killed, sexed and then dried over anhydrous calcium sulphate and preserved for their wing length to be measured as an index of size. To do this, a single wing was detached and mounted in xylene-based DPX on a microscope slide. The wing length from the distal end of the alula to the tip of the wing excluding the fringe scales was measured to the nearest 0·01 mm using an ocular micrometer under ×40 magnification.

Experiment 2

The second experiment aimed to look at the combined effects of larval competition and predation. Three larval density levels and four predation manipulation treatments were applied in a factorial experimental design. Experimental larval breeding sites were seeded with either 32, 128 or 512 first-instar larvae in the same manner as Experiment 1. Breeding sites were assigned to four predation treatments. (i) Pots left uncovered so that they could be

colonised by any predator. (ii) Pots covered by a coarse wire mesh (5 cm diameter holes) large enough to allow access to any non-vertebrate predators. (iii) Pots covered with finer mesh (1·5–2 cm in diameter holes) allowing colonisation by small but not large invertebrate predators. (iv) Pots covered by mosquito netting excluding all predators as in Experiment 1. The experiment was run six times between April and December 2011, three times each in the dry season and in the wet season. The number of times the 12 treatment combinations were replicated per run depended on the mosquito larvae available in the rearing facility: in four cases, five replicates were set up, and in two runs, there were three replicates. The breeding sites were monitored daily for the presence of pupae and predators. Predator numbers were estimated visually, and representative samples collected and identified morphologically to family level using Merritt & Cummins (1996). Mosquito pupae were collected using a sucking pipette, and their identity confirmed in the laboratory.

Statistical Methods

Data were analysed using generalised linear modelling techniques implemented in the R statistical package (R Development Core Team, URL http://www.R-project.org/). The proportion of larvae that pupated per larval breeding site was modelled using a quasi-binomial error structure to account for overdispersion. The error structure of the median time to pupation per breeding site was found to be well described by a quasi-Poisson distribution with the variance proportional to the mean. The mean wing length of the mosquitoes emerging per breeding site was normally distributed. Initial density (either log-transformed or untransformed) was treated as a continuous explanatory variable and season or block (nested within season) as fixed factors. In Experiment 2, the four predation manipulations were treated as fixed factors. Model fits were inspected graphically, and the optimum statistical models investigated by stepwise deletion with significance assessed using the appropriate test (*F*-test or likelihood ratio) for the assumed error structure.

Results

Experiment 1

There was considerable variation in survival as measured by the probability of successful pupation across the different temporal blocks (Fig. 1a). To explore whether this was due to the effect of the wet vs. the dry season, we first fitted season as a factor and then separate factors for the seven temporal blocks. The addition of both factors was significant (Table 1) with season explaining 11% of the initial deviance (the equivalent in a generalised linear model of the total variance) for one degree of freedom and blocks a further 24% for five degrees of freedom. In the wet season, survival was on average half that in the dry season with the odds of survival decreasing by a factor of 0·50 (SE range, 0·42–0·59). Thus, together, time of year and possibly other block effects explain about a third of the total variation in survival.

After controlling for block, the addition of log density was highly significant and explained a further 24% of the initial deviance (Fig. 1a; Table 1a). The form of the density dependence was nearly linear (slope, −0·75; SE, 0·09) when the log-odds of survival were plotted against log density. Increasing log density by an amount x results in a reduction in the odds of

survival by a multiplicative factor of 0·47x. The form of density dependence did not differ significantly in the dry and wet season although there was a significant block × log density interaction (though explaining only a further 5% of the initial deviance). Inspection of Fig. 1a shows this is due to mortality increasing particularly strongly with density in two blocks, for reasons that we cannot explain.

Higher larval density not only increases mortality but lengthens the time the mosquito requires to reach the pupal stage (Fig. 1c and d). Again there was substantial variation amongst the temporal blocks. The effect of season, though significant, was much less important than in the analysis of survival and explained only 4% of the initial deviance (for one degree of freedom), while block explained a further 37% for five degrees of freedom (Table 1b). After controlling for block, the addition of density significantly improved the fit of the model explaining 31% more of the initial deviance (untransformed rather than log density was used as the relationship was linear on this scale). Across the range of densities used in the experiment, the median time to pupation increased from about 5 to 9 days. There was a significant interaction between block and log density although this only explained 6% of the data for five degrees of freedom.

Mosquitoes emerging from high-density breeding sites tend to be smaller than those from low-density breeding sites. To avoid pseudoreplication, the mean wing length was analysed from mosquitoes emerging from the same larval breeding site. Males and females were analysed separately although the results were similar. Fitting season as a factor had no significant effect although there were significant block effects explaining 12% and 18% of the total variance for females and males, respectively (Table 1c & 1d). Controlling for block effects, the addition of both a linear and quadratic log density term significantly improved the fit of the model explaining together 27% (females) and 26% (males) of the total variance. In Fig. 1b, we plot the wing length of all mosquitoes as a function of density. Mosquitoes emerging from the three lower density treatments were of approximately the same size, but those from the two higher density treatments, and in particular the highest, are smaller.

Experiment 2

The experiment consisted of three density treatments crossed with four predator treatments replicated in six temporal blocks. Survival varied significantly across blocks, and there was a strong effect of density (Table 2). The average probability of pupating at a density of 32 larvae per breeding site is 0·43 (SE, 0·38–0·49), which falls to 0·32 (SE, 0·29–0·34) at a density of 128 and 0·09 (SE, 0·08–0·10) at the highest density of 512 (Fig. 2). There was a significant block by density treatment interaction (Fig. 2a), and in two blocks, low-density mortality was as high as in the most crowded treatment.

There was no significant effect of predator treatment on survival (Table 2, Fig. 2b). Predators did colonise the unenclosed breeding sites but at low densities and with very high variance: most sites had no predators but some had many. There was no significant interaction between the predator and density treatments (Fig. 2b). Survival at low densities in the open (no netting or mesh) treatments was slightly higher than in the others. In this treatment, we found some oviposition by wild *Anopheles*, which could normally be recognised because the

larvae were smaller than the experimental cohort. However, in a few cases, we recorded more pupae than larvae suggesting ovi-position early in the experiment resulting in larvae that could not be separated. The lack of difference across predation treatments in the relationship between survival and larval density shows that this was not a major factor influencing the results.

Discussion

Anopheles gambiae larvae in semi-natural breeding sites in the field experienced substantial density-dependent impairment in fitness. We found that as densities increased survival prospects worsened, the length of time required to reach the pupal stage went up, and there was a reduction in the size of the resulting adult mosquitoes. In the first experiment, we found that ~20–30% of the variation in all three measures could be explained by the density treatment although there were also significant temporal block effects. Exactly, the same thing was found in the second experiment where only survival was recorded. This experiment was designed to explore the effects of different guilds of predation on larval mosquito survival, but none were found. The results are consistent with density dependence being under- rather than over-compensatory.

The main ecological requirements of *A. gambiae* larvae were established by the 1950s (Muirhead-Thomson 1951) and have been confirmed by a series of recent studies (e.g. Gimnig *et al.* 2001; Bogh *et al.* 2003; Klinkenberg *et al.* 2003; Ye-Ebiyo *et al.* 2003; Fillinger *et al.* 2004; Koenraadt, Githeko & Takken 2004; Minakawa *et al.* 2004, 2005; Mutuku *et al.* 2006a,b; Tuno *et al.* 2006). It is found most frequently in small, relatively clean and frequently temporary sunny water bodies, without overhanging vegetation. However, as has been repeatedly stressed (Muirhead-Thomson 1951; Fillinger *et al.* 2004), *A. gambiae* has broad habitat tolerances and can be found in many different types of water body, and equally is absent from some apparently suitable pools. The design of the breeding sites used in our experiments, which we refer to as semi-natural, was a compromise between mimicking actual local breeding sites and reducing variance amongst replicates. The containers were thus round, lined with sterilised mud and contained no internal structure such as clods of mud or dead or living vegetation. They were also initiated using unsterilized local river water, mixed to ensure all sites received a similar microbial flora. The breeding sites were sunk into the earth very near to an area where *A. gambiae* naturally breed, and thus, we believe experienced a very similar microclimate.

Most accounts of the population biology of *A. gambiae* have assumed that the main source of density dependence affecting population densities is competition for resources at the larval stage, and this may be manifest through increased mortality, increased development time and decreased adult size. The evidence for this comes from laboratory experiments with *A. gambiae* and a limited number of field experiments.

Larval density dependence in *A. gambiae* has been demonstrated a number of times in the laboratory. For example, Lyimo, Takken & Koella (1992) showed that at higher larval densities development time increased, and adult size tended to be smaller although this was affected by temperature. Similarly, Takken, Klowden & Chambers (1998) used larval density

manipulation to obtain adults of different size, and Schneider, Takken & McCall (2000) showed that at high densities, *A. gambiae* sibling species competed for food resources. These experiments clearly show the potential for density-dependent larval competition, but their extrapolation to what happens in the field must be made with care. The laboratory environment is relatively benign compared with the field, which may explain why the effects of competition tend to be observed at densities that are high though not unknown in the wild. Perhaps more seriously, larval mosquitoes in the laboratory are typically fed using fish food (to generate a microbial flora), which is clearly different from what mosquitoes experience in the field. Indeed, as Gimnig *et al.* (2002) stress, we still know relatively little about exactly what microbial organisms *A. gambiae* most often consume.

There are a small number of experiments that have attempted to manipulate *A. gambiae* densities in the field of which the most important were conducted by Gimnig *et al.* (2002). We based some of our experimental methods on this study. In their main experiment, they placed between 20 and 200 first-instar larvae artificial breeding sites containing ~1 L of water, roughly comparable to the densities we used in our slightly bigger containers. They found that adults from high-density treatments were comparatively small and that they took longer to develop. However, unlike in our experiments, they did not observe a decline in survival at high densities. In a further experiment with just two density treatments, they again observed responses in adult size and development time but not survival. In this experiment, they attempted to increase the food resources for the larvae by adding 1 g of cow dung, but this had no effect on mosquito fitness.

Further data on mosquito density dependence in an unpublished PhD thesis (Njunwa 1993) have recently been re-analysed by White *et al.* (2011). Njunwa placed batches of larvae in artificial breeding sites at five densities. Mortality increased with density but then plateaued (at ~2% survival) in breeding sites with the highest number of larvae. A further experiment explored mortality when first-instar larvae were added not in a single batch, but at staggered individuals; though, the results are harder to interpret.

Our results with the few previous studies that have manipulated larval numbers in the field all point to density dependence at the larval stage being significant in the field. Observed larval densities vary greatly in natural habitats, and although comparisons are difficult to make between natural and experimental breeding sites, they do seem on occasion to reach the highest of our manipulation treatments densities (Fillinger *et al.* 2004; Mwangangi *et al.* 2006). It is also clear that larval densities can affect survival, development time and adult size, although it is puzzling that in Gimnig *et al.*'s (2002) study, mortality was not affected by the number of larvae in the breeding site. While the fitness consequences of increased mortality are obvious, the natural history of *A. gambiae* suggests that the two other responses may also be highly correlated with fitness. *A. gambiae* frequently breeds in temporary pools that dry up after rains (Muirhead-Thomson 1951), and so delayed development may indirectly lead to increased mortality (which would not have occurred in our study as we kept the water volumes in the breeding sites constant). Adult longevity in mosquitoes can at least under some circumstances be size-dependent with small individuals having low fitness and, importantly, having reduced probability to live long enough to contract, incubate and transmit pathogens (Lyimo & Koella 1992).

Our attempt to manipulate predation in the field failed. Predators did colonise the breeding sites, but the variance was so great that the statistical power to detect any differences amongst the treatments was negligible. It is possible that there was some aspect of our artificial breeding sites or their location that rendered them unattractive to predators, but we suspect that the nature of *A. gambiae* breeding sites – small temporary pools – makes their discovery by mosquito natural enemies highly haphazard. Interestingly, another member of the *gambiae* complex, *A. arabiensis* breeds in larger water bodies such as rice bodies and field studies by Service (1973, 1977) suggested both parasitism and predation to be measurably important to this species' dynamics.

For the last 50 years, the dominant conceptual paradigm for studying vector-borne diseases, in particular malaria, has been the Ross–Macdonald model (Ross 1911; Macdonald 1957; Anderson & May 1991; Smith & McKenzie 2004). The equation describing adult mosquito densities assume constant recruitment to the adult stage (or in some extensions seasonally cyclic or rainfall-driven recruitment), which implicitly assumes perfect density dependence in the larval stage. Where vector control is concentrated on killing adult mosquitoes and in particular reducing adult longevity to a level at which few survive long enough to transmit disease, this simplification of the insect's population dynamics may not be too restrictive. However, there are increasing calls today for integrated vector management (IVM) and for multipronged strategies including larval control and habitat modification (Yohannes *et al.* 2005; Killeen *et al.* 2006). There is also an exciting range of new vector-control measures under research employing techniques from modern molecular biology to suppress mosquito populations often by targeting juvenile stages (Burt 2003; Sinkins & Gould 2006). To implement IVM or novel genetic methods efficiently, better models of mosquito-vector population dynamics are required incorporating richer information about larval biology and in particular important ecological processes such as density dependence. Although we have concentrated here on malaria vectors, the same is true of other major mosquito vectors, for example there are only a handful of studies that have looked at density dependence in the field for the yellow fever and dengue virus vector, *Aedes aegyptii* (Southwood *et al.* 1972; Dye 1984; Legros *et al.* 2009; Walsh *et al.* 2011). Given that *A. gambiae* can claim to be the most dangerous species of animal on earth (in terms of the scale of deaths and morbidity it causes), and a species whose molecular biology is perhaps more studied than any animal except the classic model organisms, it is salutary and worrying how little we still know about its population ecology.

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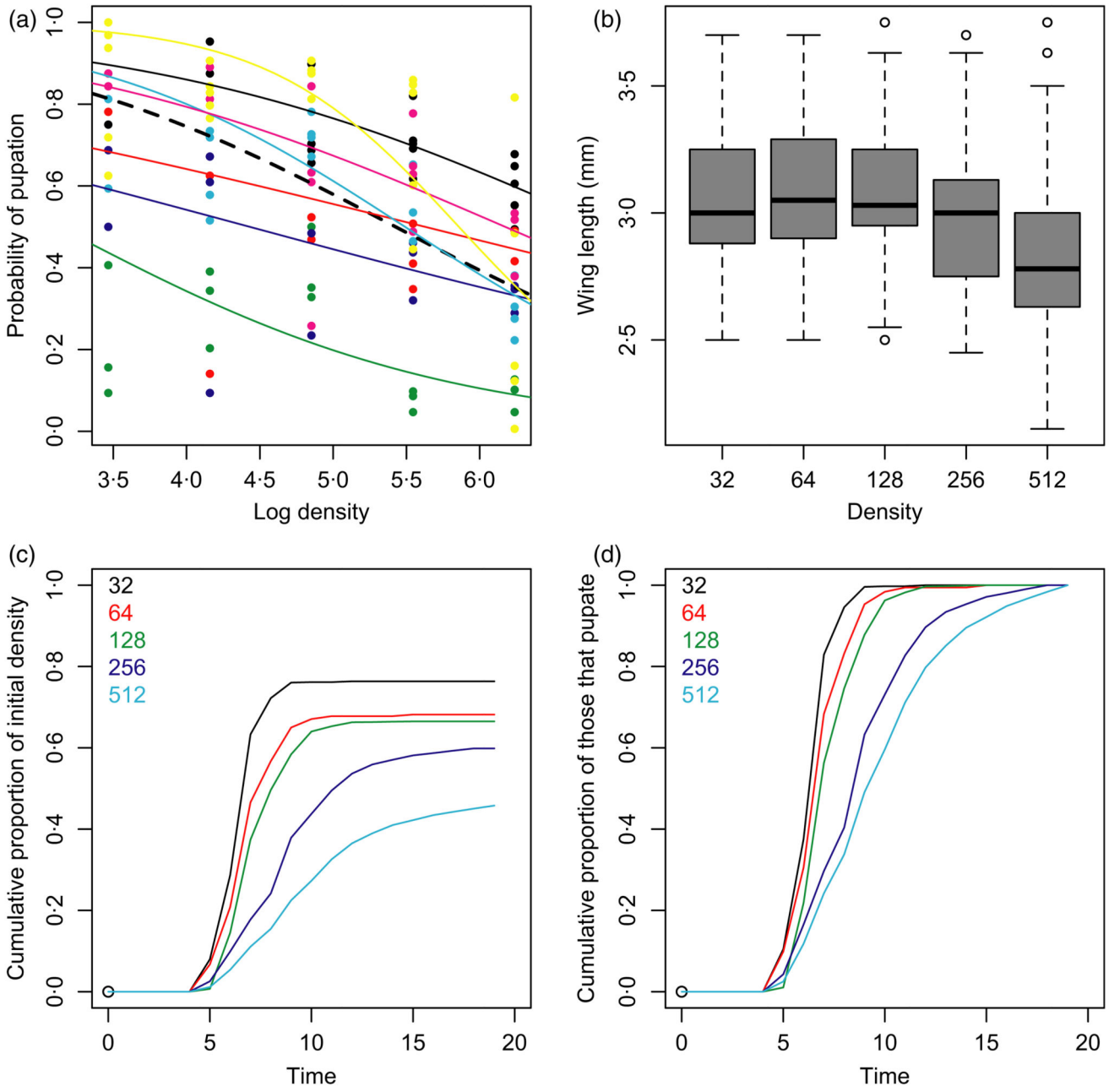

Fig. 1. The effects of larval density on mosquito fitness (Experiment 1).

(a) The probability of pupation as a function of the log of initial density. The raw data are shown with the estimated block mean effects in different colours (dry season: black, red, yellow; wet season: green, blue, cyan, magenta). The overall mean pupation rate as a function of density is represented by the heavy dashed line. (b) Box-whisker plot of wing length as a function of density, all blocks combined. The box shows the inter-quartile range with the median represented by a line; the whiskers show the full range of data except for outliers represented by circles. (c) The cumulative proportion of mosquitoes that pupate in the different density treatments as a function of time after the larvae were added to the

experimental breeding sites. (d) As panel c but here the standardised cumulative proportion of all those mosquitoes that will eventually pupate are plotted as a function of time to make visual comparison between the different density treatments easier.

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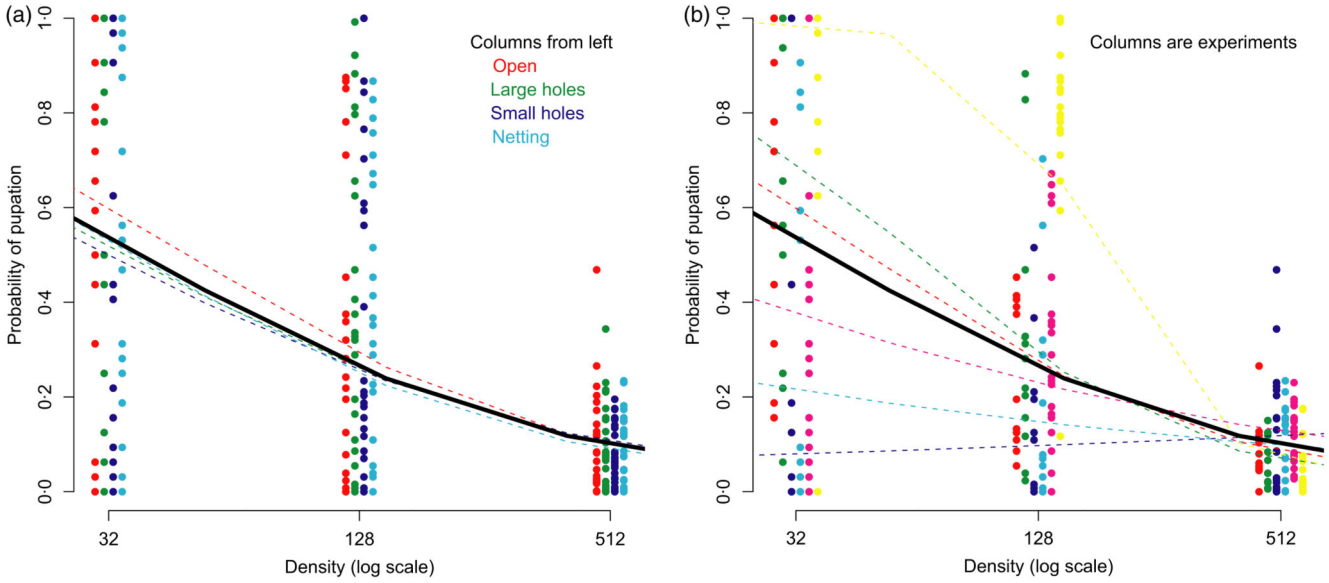

Fig. 2. The probability of pupation as a function of initial population size in the predation experiment. The raw data for different breeding sites are shown as dots.

In (a), the four different predation treatments are shown as separate columns with dotted lines treatments means and the heavy continuous the overall mean. In (b), data from the six replicate experiments are shown in the columns along with experiment and overall fitted means.

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Table 1

Analysis of deviance of the different components of mosquito fitness measured in Experiment 1. Resid. dev and % dev explained are residual deviance and percentage deviance explained, where deviance is equivalent to sum of squares in an analysis of variance

	d.f.	Resid. dev	*F*-value	*P*-value	% dev explained
(a) Pupation probability					
Intercept only	134	7210			
Add season	133	6401	38.0	< 0.0001	11
Add block	128	4643	16.5	< 0.0001	24
Add log initial density	127	2940	79.9	< 0.0001	24
Add block*density	121	2582	2.8	0.014	5
(b) Time to pupation					
Intercept only	134	53.4			
Add season	133	51.5	18.8	< 0.0001	4
Add block	128	31.8	40	< 0.0001	37
Add initial density	127	15	171.3	< 0.0001	31
Add block*density	121	11.7	6.5	< 0.0001	6
(c) Wing length, females					
Intercept only	72	4.05			
Add season	71	4.05	0.01	0.91	<1
Add block	66	3.58	2.45	0.042	12
Add initial density (linear)	65	2.67	23.75	< 0.0001	(linear and quadratic) 27
Add initial density (quadratic)	64	2.47	5.14	0.027	
(d) Wing length, males					
Intercept only	72	3.99			
Add season	71	3.91	2.42	0.12	2
Add block	66	3.21	4.26	0.002	18
Add initial density (linear)	65	2.42	24.07	< 0.0001	(linear and quadratic) 26
Add initial density (quadratic)	64	2.17	7.57	0.008	

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Table 2

Analysis of deviance of the effects of density and predation treatments in larval mosquito survival in Experiment 2. Column headings as in Table 1

	d.f	Resid. Dev	*F*-value	*P*-value	% dev explained
Pupation probability					
Intercept only	311	19584			
Add block	306	18670	6·15	< 0·0001	5
Add density	304	13112	93·31	< 0·0001	28
Add density × block	294	8685	14·89	< 0·0001	23
Add predation	291	8624	0·68	0·56	<1